Supporting Information

Li *et al*. 10.1073/pnas.0807265105

SI Materials and Methods

Confocal Microscopy. Approximately 1×10^5 primary thioglycollate-elicited macrophages from RNaseL$^{-/-}$ mice were plated into each chamber of glass bottomed, 8-well chamber slides (Nunc). Cells were infected with BA spores at a MOI of ≈5 at 37°C for 5 h, then washed with PBS, and fixed with 4% *p*-formaldehyde for 12 min at room temperature. Cells were blocked and permeabilized for 30 min with PBST (PBS, 1% BSA, 1% normal donkey serum, 0.3% Triton X-100) at room temperature. CatE was visualized by immunofluorescence using polyclonal antibody against the C terminus of mouse Cathepsin E (AbCam), followed by Cy3-conjugated donkey anti-rabbit IgG (Jackson). CD11b was detected using monoclonal IgG2b antibody raised against mouse CD11b (Integrin αM, Mac-1α; eBiosciences), followed by a secondary Cy-5- conjugated donkey anti-rat IgG (Jackson). BA spores were visualized by addition of a human monoclonal antibody against protective antigen selected for its toxin-neutralizing activity (1), followed by Alexa-Flour 488 goat anti-human IgG (Molecular Probes). Nucleic acid was detected using DAPI (4′,6′-diamidino-2-phenylindole) stain. Coverslips were mounted on slides using a DABCO-based anti-fading fluorescent mounting medium and viewed with an Olympus FluoView 500 confocal microscope (60x, NA 1.4 objective) fitted with standard excitation and emission filters for the visualization of UV, Cy2, Cy3, and Cy5.

RNase-L-Mediated rRNA Cleavage. rRNA cleavage was analyzed by formaldehyde-agarose gel electrophoresis of total RNA, and RNA was detected by ethidium bromide staining.

1. Vitale L, *et al*. (2006) Prophylaxis and therapy of inhalational anthrax by a novel monoclonal antibody to protective antigen that mimics vaccine-induced immunity. *Infect Immun* 74:5840–5847.

2. Li XL, *et al*. (2007) Post-transcriptional regulation of RNase-L expression is mediated by the 3′-untranslated region of its mRNA. *J Biol Chem* 282:7950–7960.

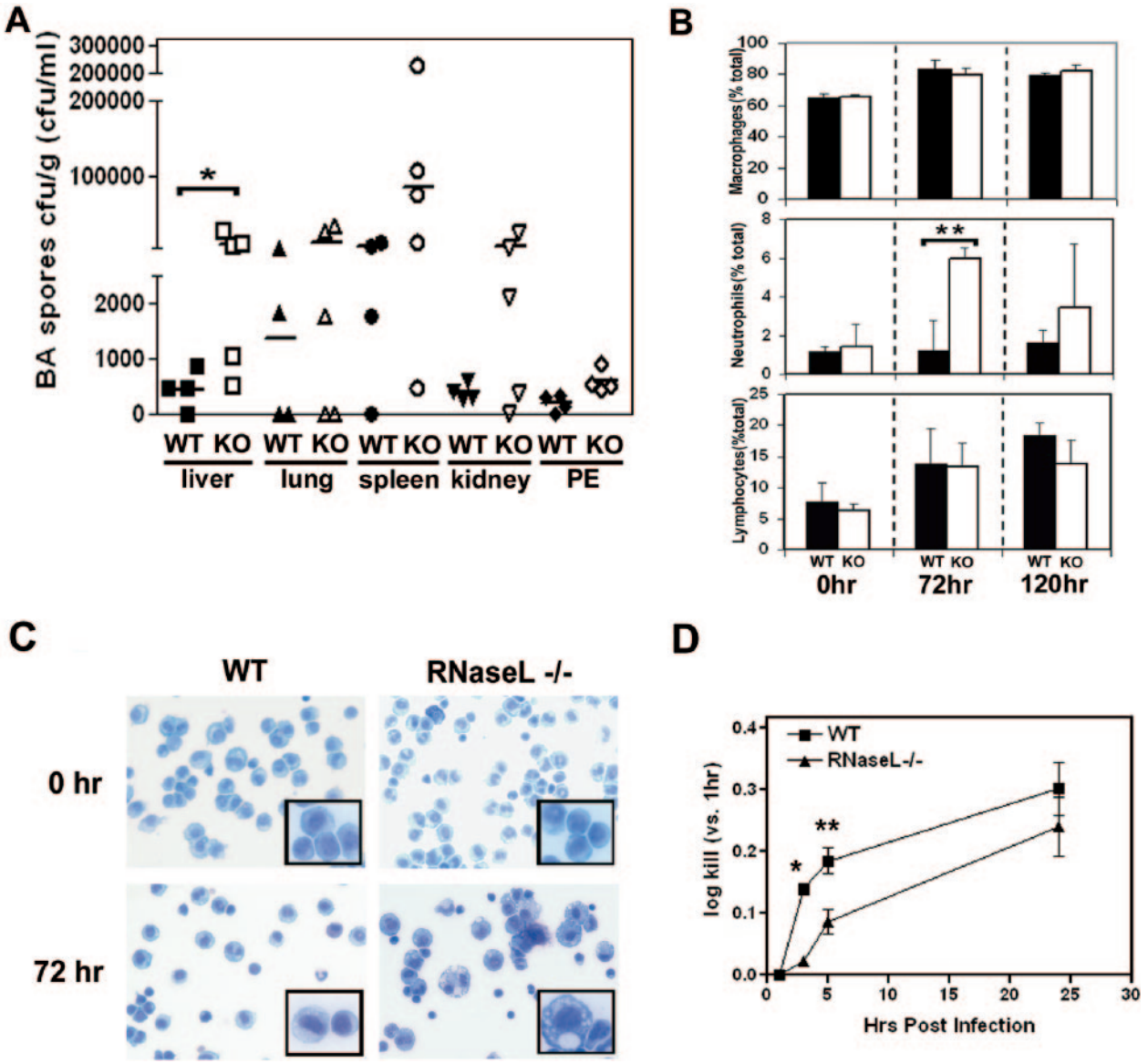

Fig. S1. Immune response to BA is impaired in RNase-L$^{-/-}$ mice. (*A*) Mice were inoculated with 4×10^5 cfu BA Sterne spores and bacterial titers in organs (cfu/g), and peritoneal exudates (PE), and blood (cfu/ml) were determined at 72 hpi. Lines indicate mean values, and (*) denotes $P < 0.05$. (*B*) Macrophages, neutrophils, and lymphocytes in peritoneal fluid at the indicated hours post BA infection (hpi) are expressed as the average of the percentage of a given cell type in ≥400 total cells counted/mouse from four independent mice +/- s.d. (*) denotes $P < 0.05$. (*C*) At the indicated times following infection with 4×10^5 cfu BA Sterne spores i.p., cells in the peritoneal fluid were isolated and stained; representative fields are shown at 200x and 400x (inset) magnification. D. Peritoneal macrophages from RNase-L-/- and WT mice were infected with BA spores (MOI = 0.2) for the indicated times, and the viable spores were quantified, and expressed as the log reduction in cfu as compared to the 1 h value; (*) signifies $P < 0.05$; (**) signifies $P < 0.001$.

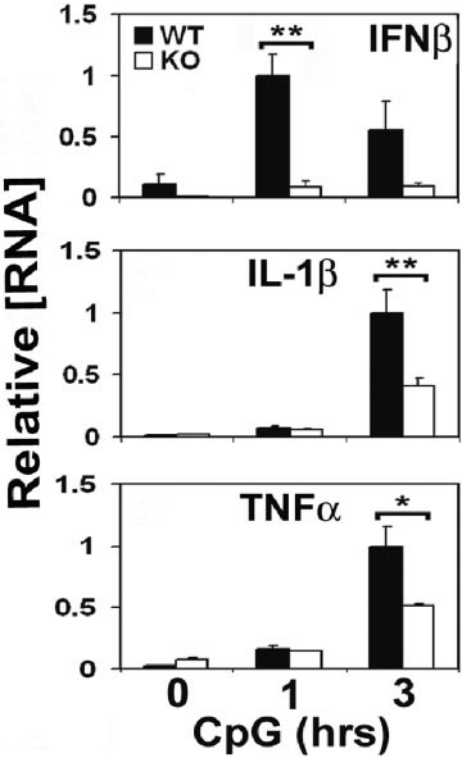

Fig. S2. Cytokine induction by CpG is impaired in RNase-L$^{-/-}$ macrophages. Cytokine expression was measured by qRTPCR following CpG (25 μg/ml) treatment for the indicated times. The data shown is representative of 2–5 independent experiments; error bars, sd; (*) signifies $P < 0.05$; (**) signifies $P < 0.001$.

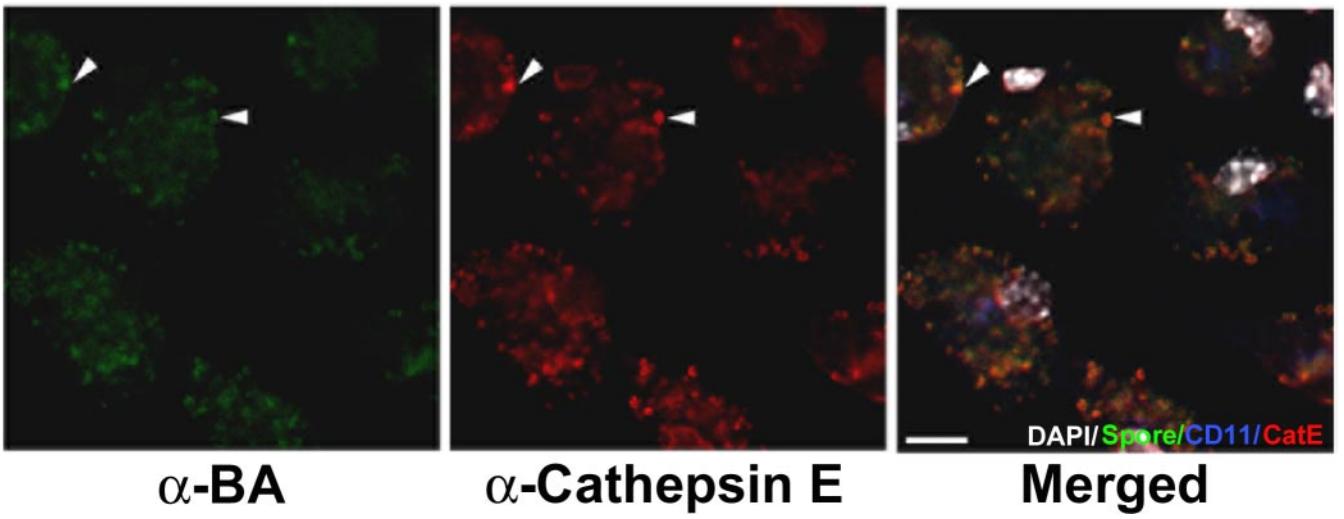

Fig. S3. CatE colocalizes with BA spores in macrophages. RNaseL$^{-/-}$ macrophages were infected for 5 h with Sterne strain of BA spore (MOI = 5). Cells were fixed and immunostained for spores (green), CatE (red), macrophage marker (CD11b, blue), and nucleic acid (DAPI, white). Signals from BA (*Left*) and CatE (*Center*) were merged to detect colocalization (*Right*); examples of colocalized signals are indicated by arrowheads). The colocalization shown is representative of three independent experiments. Scale bar = 10 μm.

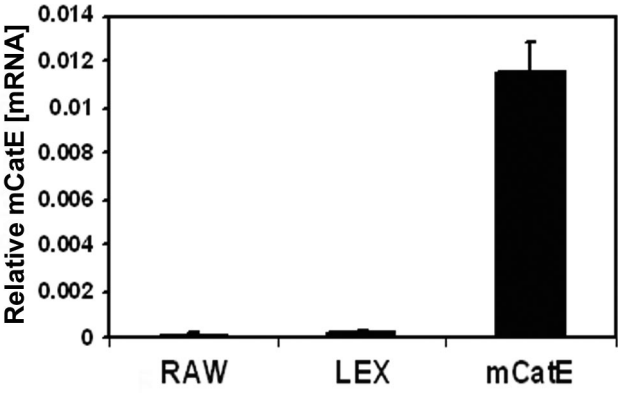

Fig. S4. CatE expression in parental RAW264.7 cells, and following stable transduction with lentiviral vector (LEX3) or catE construct, was measured by qRTPCR.

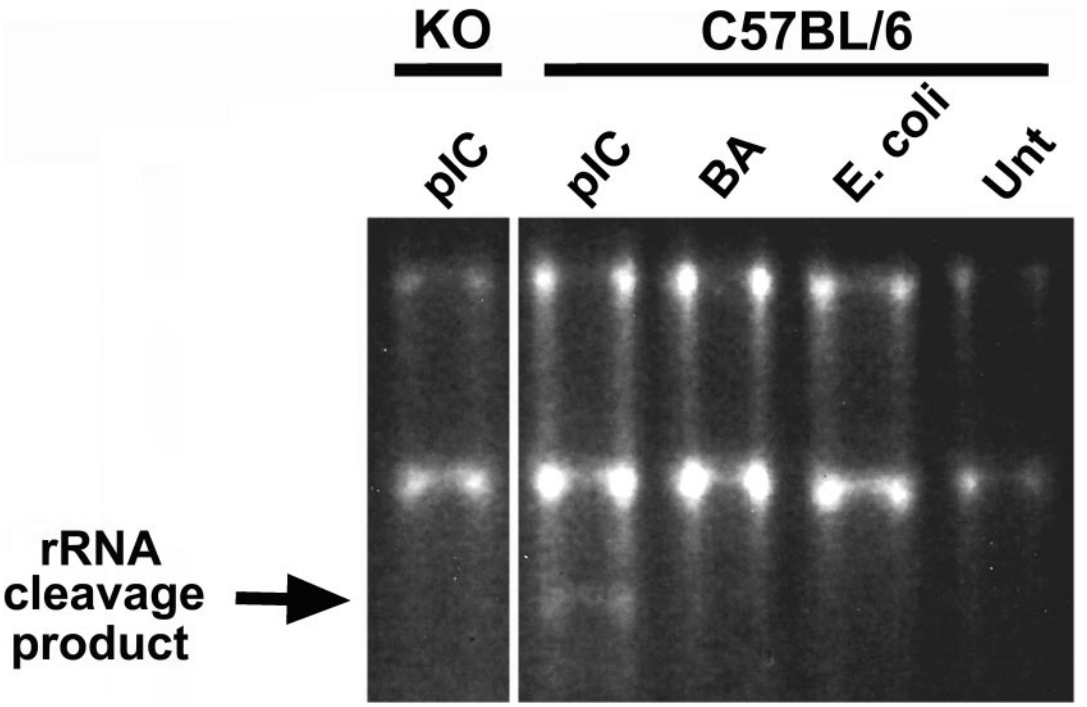

Fig. S5. RNase-L mediated rRNA cleavage is not detectable following bacterial infection. Total RNA isolated from WT (C57BL/6) or RNase-L$^{-/-}$ (KO) macrophages following infection with *E. coli* (20 h, MOI = 5) or BA (20 h, MOI = 2) as indicated. Treatment with dsRNA (8 h, 25 μg/ml) was used to activate OAS as a positive control for rRNA cleavage; the absence of rRNA cleavage in dsRNA treated RNase-L$^{-/-}$ macrophages demonstrated the RNase-L dependence of this activity. Note that the space between this lane and the remainder of the gel is to indicate that it was repositioned in the figure to permit a direct comparison of rRNA cleavage in adjacent WT and RNase-L$^{-/-}$ macrophage samples. RNA was analyzed (15 μg/lane) for rRNA cleavage by electrophoresis on formaldehyde-agarose gels; RNA was stained with ethidium bromide, and visualized with UV light.

Table S1. Differential induction of nonimmune genes

Gene title	Symbol	KO unt vs. WT unt	KO 8 h vs. WT 8 h	KO 8 h vs. KO unt	WT 8 h vs. WT unt	*P* value	KO vs. WT fold induction at 8 hpi
MIT, microtubule interacting and transport, domain containing 1	Mitd1	1.01	−2.13	1.95	4.2	< 0.001	−2.16
BAH domain and coiled-coil containing 1	Bahcc1	1.01	−2.36	1.56	3.73	< 0.001	−2.39
Prostaglandin-endoperoxide synthase 2	Ptgs2	1.16	−1.74	2.53	5.1	0.001	−2.01
Spindle assembly 6 homolog (*C. elegans*)	Sass6	1.05	−1.84	1.75	3.39	0.001	−1.93
EGL nine homolog 3 (*C. elegans*)	Egln3	−1.68	1.13	−1.51	−2.87	0.004	1.91
serine (or cysteine) peptidase inhibitor, clade B, member 2	Serpinb2	1.84	−1.06	−2.53	−1.29	0.013	−1.96
Proteolipid protein 2	Plp2	1.15	−2.22	−2.38	1.07	0.028	−2.55
Phosphoenolpyruvate carboxykinase 2 (mitochondrial)	Pck2	−1.4	1.51	−1.23	−2.6	< 0.001	2.11
Trichorhinophalangeal syndrome I (human)	Trps1	−1.64	1.18	2.17	1.13	0.004	1.93
Mitogen-activated protein kinase 1	Mapk1	1.11	−1.83	−2.35	−1.16	0.016	−2.03

Differential induction of nonimmune genes at 8 hpi with BA spores of WT and RNase-L$^{-/-}$ macrophages; microarray data was filtered as described in Table 1.

Table S2. Number of probe sets identified as significantly differentially modulated

Comparison	Number of probe sets
KO 2 h vs. KO unt	1691
KO 8 h vs. KO unt	469
WT 2 h vs. WT unt	936
WT 8 h vs. WT unt	755
KO unt vs. WT unt	6
KO 2 h vs. WT 2 h	0
KO 8 h vs. WT 8 h	4
KO 2 h vs. WT unt	1657
KO 8 h vs. WT unt	567

Microarray analysis results. Pairwise comparisons between treatments of over 45,000 gene fragments (probe sets) produced a total of 2,382 probe sets with a *P* value of less than 0.05 and an absolute ratio of 1.3 for at least one comparison. WT, C57Bl/6; KO, RNase-L−/−.

Table S3. mRNA half life (hrs)

	WT	KO
catE	1.3	16
TNFα	0.42	0.64
IL-1β	0.42	0.53
TLR3	10.7	11.2
c-fos	0.51	0.32

RNase-L-dependent regulation of mRNA stability. The half lives of the indicated mRNAs in WT and RNase-L$^{-/-}$ macrophages were calculated as described (2).

Table S4. qPCR primers

Mouse	
CatE	forward; 5′ TTGACACCGGTTCATCCAACCTCT-3′, reverse; 5′ ATGGATGGAATACTGGGTGTGCCT-3′.
LAMP1	forward; 5′ TAGTGCCCACATTCAGCATCTCCA-3′, reverse; 5′ TTCCACAGACCCAAACCTGTCACT-3′.
LAMP2	forward; 5′ AGAAGACCAAACTCCCACCACTGT-3′, reverse; 5′ AGTTGGAGTTGGAGTGGGTGTTGA-3′.
c-fos	forward; 5′ ATCCTTGGAGCCAGTCAAGAGCAT-3′, reverse; 5′ TCCCAGTCTGCTGCATAGAAGGAA-3′.
TLR3	forward; 5′ ACCTTTCCGCCCTCTTCGTAACTT-3′, reverse; 5′ AGACCCTCCAGCAAGTCCTCATTT-3′.
GAPDH	forward; 5′ TGTGATGGGTGTGAACCACGAGAA-3′, reverse; 5′ GAGCCCTTCCACAATGCCAAAGTT-3′.
IFN-β	forward; 5′ CACTTGAAGAGCTATTACTGGAGGG-3′, reverse; 5′ CTCGGACCACCATCCAGG-3′.
IL-1β	forward; 5′ TGGAGAGTGTGGATCCCAAGCAAT-3′, reverse; 5′ TGTCCTGACCACTGTTGTTTCCCA-3′.
TNF-α	forward; 5′ AGCCGATGGGTTGTACCTTGTCTA-3′, reverse; 5′ TGAGATAGCAAATCGGCTGACGGT-3′.

Sequences of primers used in this study.